

REPORTS

Multiple Primary Cancers in Families With Li-Fraumeni Syndrome

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Background: Li-Fraumeni syndrome is a dominantly inherited disorder characterized by early-onset breast cancer, sarcomas, and other cancers in children and young adults. Members of families with this syndrome also develop multiple primary cancers, but the frequency is unknown. To approach this issue, we quantified the incidence of second and third primary cancers in individuals from 24 Li-Fraumeni kindreds originally diagnosed with cancer during the period from 1968 through 1986. **Methods:** The relative risk (RR) of subsequent cancers and 95% confidence intervals (CIs) were calculated by use of population-based incidence data from the Connecticut Cancer Registry. Kaplan-Meier analysis was used to determine the cumulative probability (\pm standard error) of subsequent cancers. **Results:** Among 200 Li-Fraumeni syndrome family members diagnosed with cancer, 30 (15%) developed a second cancer. Eight individuals (4%) had a third cancer, while four (2%) eventually developed a fourth cancer. Overall, the RR of occurrence of a second cancer was 5.3 (95% CI = 2.8–7.8), with a cumulative probability of second cancer occurrence of 57% ($\pm 10\%$) at 30 years after diagnosis of a first cancer. RRs of second cancers occurring in families with this syndrome were 83.0 (95% CI = 36.9–187.6), 9.7 (95% CI = 4.9–19.2), and 1.5 (95% CI = 0.5–4.2) for individuals with a first cancer at ages 0–19 years, 20–44 years, and 45

years or more, respectively. Thirty (71%) of 42 subsequent cancers in this group were component cancers of Li-Fraumeni syndrome. **Conclusions:** Compared with the general population, members of Li-Fraumeni syndrome families have an exceptionally high risk of developing multiple primary cancers. The excess risk of additional primary cancers is mainly for cancers that are characteristic of Li-Fraumeni syndrome, with the highest risk observed for survivors of childhood cancers. Cancer survivors in these families should be closely monitored for early manifestations of new cancers. [J Natl Cancer Inst 1998;90:606–11]

Li-Fraumeni syndrome (LFS), an autosomal-dominant disorder, features the occurrence of breast cancer in young women and of soft tissue sarcomas, osteosarcomas, brain tumors, acute leukemias, and adrenocortical tumors in children and young adults (1–7). Germline mutations in the p53 tumor suppressor gene (also known as TP53) have been identified in approximately one half of LFS families in the literature (8–12). Our follow-up studies of LFS families revealed that new cancers, including multiple primary cancers, continued to develop among at-risk relatives (13). The current study quantifies the frequency of multiple primary cancers in these kindreds.

Subjects and Methods

Study Population

Study subjects are members of 24 LFS kindreds who were enrolled in the Cancer Family Registry in the Division of Cancer Epidemiology and Genetics, National Cancer Institute, during the period from 1968 through 1986 (1,2). Initial informed consent of some families utilized standard procedures that antedated institutional review boards, whereas subsequent studies were performed with written consent on protocols approved by the institutional review board of the Dana-Farber Cancer Institute. We recontacted family members to identify cancers, births, and deaths that occurred after the last systematic follow-up in 1986. A total of 1004 blood

relatives in the affected lineages were enumerated for this study.

Diagnoses of cancer were based on available medical records, pathology reports, and death certificates. Written consent to review medical records was obtained from living subjects or next of kin of decedents. The diagnosis of multiple primary cancers was based on findings of malignant neoplasms of different histologic types or primary anatomic sites. Multiple primary breast cancers were diagnosed when these cancers differed in histology or occurred more than 5 years apart without metastases to other sites. Unconfirmed cancers, carcinomas of the skin, and *in situ* carcinomas were excluded from analysis. The majority of unconfirmed cancers were diagnosed before 1985. Available specimens from affected members of 16 of the 24 families were analyzed for germline p53 mutations. Eight (50%) of these 16 kindreds had germline p53 mutations (8,9). No blood specimens were available from affected members of the remaining eight families because of cancer mortality.

Treatment records of patients with multiple cancers were reviewed for information regarding types of treatment (surgery, chemotherapy, and radiation therapy), specific chemotherapeutic agents and doses used, and radiation fields and doses. Treatment data were unavailable for patients whose cancer diagnosis was based on death certificates or pathology reports only.

Statistical Methods

Analyses were performed on all family members with confirmed cancers and on subgroups on the basis of age at diagnosis and type of first cancer. Person-years of observation for second cancers extended from the date of first cancer diagnosis to the date of second cancer diagnosis, death, loss to follow-up, or close of the study in October 1995. Observed numbers of cancers were compared with expected numbers estimated by multiplying appropriate person-years at risk by age-, sex-, and calendar

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year-specific incidence rates for all primary cancers in the state of Connecticut (i.e., on the basis of data from the Connecticut Cancer Registry) (14–16). Relative risks (RRs) of second cancers were the ratios of observed versus expected numbers of cancers, assuming a Poisson distribution for the number of second cancers in the LFS cohort. The Dean Score test was used to test for Poisson overdispersion of the age-specific counts stratified by 5-year calendar periods (17). The asymptotic 95% confidence intervals (CIs) were computed, adjusting for overdispersion as measured by Pearson's χ^2 statistics (18). The cumulative probability of second cancers and the standard errors (SEs) were estimated by Kaplan–Meier analysis and Greenwood's formula (19). Differences in the cumulative probability of cancer on the basis of age at first cancer diagnosis and cancer type were evaluated by Mantel–Haenszel logrank tests (20). The cumulative probability of third cancers occurring among those with double primary cancers was determined in the same manner.

The possibility of selective ascertainment of families with individuals who had multiple cancers prompted a subset analysis of living subjects who did not have second cancers at initial ascertainment. The period of observation for second cancers in these patients started from the date of family ascertainment for case subjects previously diagnosed with cancer and the date of first cancer diagnosis for those who had been cancer free. Criteria for withdrawal from observation were unchanged. Of 85 case subjects excluded from the subgroup analysis, 77 died and eight had developed multiple primary cancers before the ascertainment date.

Results

Two hundred cancer patients (96 males and 104 females) in the 24 families were eligible for study (Table 1). These 200 patients accumulated 1142 person-years

of follow-up before diagnosis of second primary cancer (30 patients), death (120 patients), loss to follow-up (two patients), or study closure (48 patients). The first cancers in 140 individuals (70%) were diagnosed before age 45 years, including 62 diagnosed within the first two decades of life. There were 140 cancers (70%) that were characteristic of LFS, i.e., breast cancers (45 women), soft tissue sarcomas (34 patients), osteosarcomas (25 patients), brain tumors (20 patients), leukemias (11 patients), and adrenocortical carcinomas (five patients). In later life, family members tended to develop cancers of the lung (13 patients), colon (seven patients), and pancreas (seven patients), as well as malignant lymphomas (seven patients).

The 30 individuals from the LFS families with a second cancer occurrence developed a total of 72 primary cancers (Table 2). The intervals between diagnosis of the first and second primary cancers ranged from 1 to 27 years (median, 6 years). Eight patients had a third cancer, and four of them eventually developed a fourth cancer. The neoplasms featured in LFS accounted for 54 (75%) of these 72 cancers, including 24 breast cancers and 22 sarcomas.

Kaplan–Meier analysis showed a cumulative second cancer probability of 57% ($\pm 10\%$ [\pm SE]) at 30 years of follow-up (Fig. 1, A). The cumulative probability was higher among 34 patients who ini-

tially had soft tissue sarcomas, i.e., 64% ($\pm 16\%$) at 20 years and 100% at 30 years of follow-up. On the basis of the 30 patients with a second primary cancer, the cumulative probability of a third cancer was 38% ($\pm 12\%$) at 10 years after the diagnosis of a second cancer (Fig. 1, B).

Subgroup analysis of the 115 cancer survivors (56 males and 59 females) who were free of a second cancer at initial ascertainment showed that 23 subsequently developed second cancers. Their cumulative probability of developing second cancers was 54% ($\pm 11\%$) at 25 years of follow-up, which is comparable to the estimate for the entire series.

The age-adjusted incidence rate of second cancer in the 24 LFS families (2.6 per 100 person-years) exceeded the expected cancer rate for the general population (RR = 5.3; 95% CI = 2.8–7.8) (Table 3). The rate was highest among those with cancer initially diagnosed before 20 years of age (3.2 per 100 person-years) and declined with age. Consequently, RRs of second cancer differed markedly by age at first cancer diagnosis (Table 3). Patients with the cancers featured in LFS did not have a higher incidence of second cancers when compared with the incidence among those with other cancers (data not shown). Patients in families with a germline p53 mutation did not have a higher incidence of second cancers when compared with the incidence among those in families without a known p53 mutation (data not shown).

Treatment records for 27 of the 30 patients who had multiple primary cancers showed that nine had received radiotherapy for their first cancer (five also had chemotherapy), three had chemotherapy only, and 15 had neither treatment. Most irradiated patients received megavoltage cobalt-60 \times rays (range, 35–70 Gy). Six irradiated patients (Nos. 1, 4, 5, 12, 16, and 30) developed a total of eight solid tumors within the radiation field at 3–22 years after treatment for the first cancer (median, 11 years) (Table 2). In addition, the radiation field for the third cancer in one patient (No. 12) encompassed the site of her fourth cancer 7 years later. One other patient (No. 24) developed acute leukoerythroblastic leukemia 2 years after treatment for a brain tumor with carmustine and cranial irradiation, a known leukemogenic regimen (21,22).

Table 1. Tumor types and ages at diagnosis of 200 first cancers in Li–Fraumeni syndrome family members*

First cancer	No. of patients with cancer (No. with second cancer) by age at first cancer diagnosis			
	0–19 y	20–44 y	≥ 45 y	All ages
Cancers featured in Li–Fraumeni syndrome				
All types	55 (9)	64 (13)	21 (2)	140 (24)
Breast cancer	0 (0)	33 (9)	12 (1)	45 (10)
Soft tissue sarcoma	18 (5)	11 (3)	5 (1)	34 (9)
Osteosarcoma	17 (3)	7 (0)	1 (0)	25 (3)
Brain tumor	9 (0)	10 (1)	1 (0)	20 (1)
Leukemia	7 (1)	2 (0)	2 (0)	11 (1)
Adrenocortical carcinoma	4 (0)	1 (0)	0 (0)	5 (0)
Other cancers†	7 (1)	14 (1)	39 (4)	60 (6)
Total cancers	62 (10)	78 (14)	60 (6)	200 (30)

*Based on 200 first cancers in 24 Li–Fraumeni syndrome kindreds identified during the period from 1968 through 1986. Study subjects were enrolled in the Cancer Family Registry, Division of Cancer Epidemiology and Genetics, National Cancer Institute.

†Lung cancer (13), lymphoma (7), colon cancer (7), pancreatic cancer (7), cancer of the uterus/ovaries (6), prostate cancer (5), kidney cancer (2; renal cell carcinoma and Wilms' tumor), cancer of esophagus (2), stomach cancer (2), bladder cancer (2), and neuroblastoma, gallbladder cancer, liver cancer, thyroid cancer, laryngeal cancer, cancer of the thorax, and skin cancer (melanoma) (1 each).

Table 2. Multiple primary cancers in 30 Li–Fraumeni syndrome family members diagnosed with a second cancer

Patient No.	Sex*	Sequence of tumor types (age at diagnosis in years)†			
		First	Second	Third	Fourth
1	M	SS (1)	SS‡,§, (23)		
2	F	SS (2)	Brain (6)		
3	F	SS (2)	Breast (29)		
4	M	SS (4)	OS§ (15)		
5	M	SS (12)	SS§, (27)		
6	F	SS (24)	Breast (34)		
7	F	SS (28)	Breast (32)		
8	F	SS (35)	SS (42)	Breast (48)	
9	M	SS (50)	Lymphoma (51)	Melanoma (53)	
10	M	OS (6)	SS‡ (17)		
11	M	OS (14)	Brain (26)		
12	F	OS (16)	SS§ (19)	Breast (29)	Breast‡,§, (36)
13	F	Breast (22)	Thyroid (30)	Breast (34)	Ovary (50)
14	F	Breast (24)	Gastric (40)		
15	F	Breast (25)	Ovary (29)	Brain (30)	
16	F	Breast (30)	Breast (36)	Mesothelioma§ (40)	SS§ (41)
17	F	Breast (32)	Breast (47)		
18	F	Breast (33)	Breast (35)		
19	F	Breast (33)	Breast (42)		
20	F	Breast (39)	Pancreas (58)	Breast (60)	
21	F	Breast (42)	Breast (46)		
22	F	Breast (57)	Breast (59)		
23	M	Leukemia (2)	Leukemia‡ (11)		
24	F	Brain (26)	Leukemia (28)		
25	M	Kidney (15)	SS (16)		
26	M	Lung (55)	SS (62)	Lung‡ (64)	Lymphoma (65)
27	M	Larynx (35)	Lung‡ (39)		
28	F	Ovary (68)	Leukemia (71)		
29	F	Pancreas (47)	Bladder (53)		
30	M	Prostate (62)	SS§ (66)		

*M = male; F = female.

†Cancer classification: SS = soft tissue sarcoma; OS = osteosarcoma. All others except brain tumors were carcinomas unless otherwise specified.

‡Histologically different from the previous cancer(s).

§Tumors occurred in the previous radiation field.

||See text for criteria to determine multiple primary cancers.

Discussion

Members of families with inherited cancer syndromes such as LFS tend to develop multiple primary cancers at early ages (2,23–26). This prospective study examined the frequency of multiple primary cancers in members of 24 LFS kindreds identified up to three decades ago. Thirty of 200 family members with cancer developed multiple primary cancers. Kaplan–Meier analysis showed a 57% cumulative probability of second cancer at 30 years after diagnosis of the first cancer. Third cancers developed at an even higher rate, although the number of patients with a third cancer was small. Most neoplasms in these families were component cancers of LFS, suggesting that inherited susceptibility was the major predisposing factor.

Cancer incidence rates are low among children in the general population and rise steadily with increasing age. In contrast,

rates of second cancer in our series were highest among childhood cancer survivors, who had an 83-fold excess risk (Table 3); however, no excess risk was found in family members with first cancers after age 45 years (Table 3), suggesting that late-onset cancers among family members might be due to chance or factors other than inherited predisposition. The RRs of second cancers were similar among patients whose first cancers are typical of LFS versus those with other neoplasms. This finding raises the possibility that some of these other neoplasms are rare manifestations of LFS (11).

Somatic mutations in the p53 gene are found in a high proportion of human cancers, whereas germline mutations are rare (27,28). Inherited p53 gene mutations have been detected in approximately one half of LFS kindreds and rarely in young patients with multiple primary cancers (29–34). Within the 24 families whom we

studied, the increased incidence of second cancers was not associated with having a germline p53 gene mutation. Families with normal p53 alleles might have germline mutations in other highly penetrant genes that produce an autosomal-dominant pattern of similar cancers. These genes can be sought by linkage studies of p53-negative families and molecular analyses. Candidate germline mutations may be in genes involved in the p53 signal transduction pathway.

Ionizing radiation is a known risk factor for virtually all cancers except chronic lymphocytic leukemia (35,36). Its carcinogenic effects are dose dependent, and high-dose radiotherapy can contribute to development of second cancers (36–41). Sensitivity to radiation-induced cancers has been reported in clinical studies of patients with germline mutations in the p53 gene and in p53-deficient mice (42–44). In our study, available data on treatment of the first cancers suggest that radiotherapy contributed to eight subsequent solid tumors in six patients and to acute leukemia in a seventh patient who also received chemotherapy. The 3- to 22-year interval between radiation treatment and solid tumor development is consistent with the latent periods for radiation carcinogenesis (37,45). The latent period, which is shorter for radiation leukemogenesis, was 2 years in our patient with secondary leukemia after radiotherapy and carmustine chemotherapy. These findings parallel our observation that radiotherapy further increases the risk of second cancer among retinoblastoma patients with germline RB1 gene mutations (23).

Our study design may have selected for LFS families known to have multiple primary cancers. Consequently, a subset analysis was restricted to second cancers that developed after initial ascertainment of the kindreds. A similar risk estimate was found, suggesting that substantial selection bias is unlikely. Overestimation may have resulted by including 15 patients with multiple sarcomas or bilateral breast cancers. However, five of these patients had third cancers as additional manifestations of their susceptibility to multiple primary cancers. Calculation of risk of second cancer also excluded unconfirmed cancers and applied histologic, anatomic, and temporal criteria for diag-

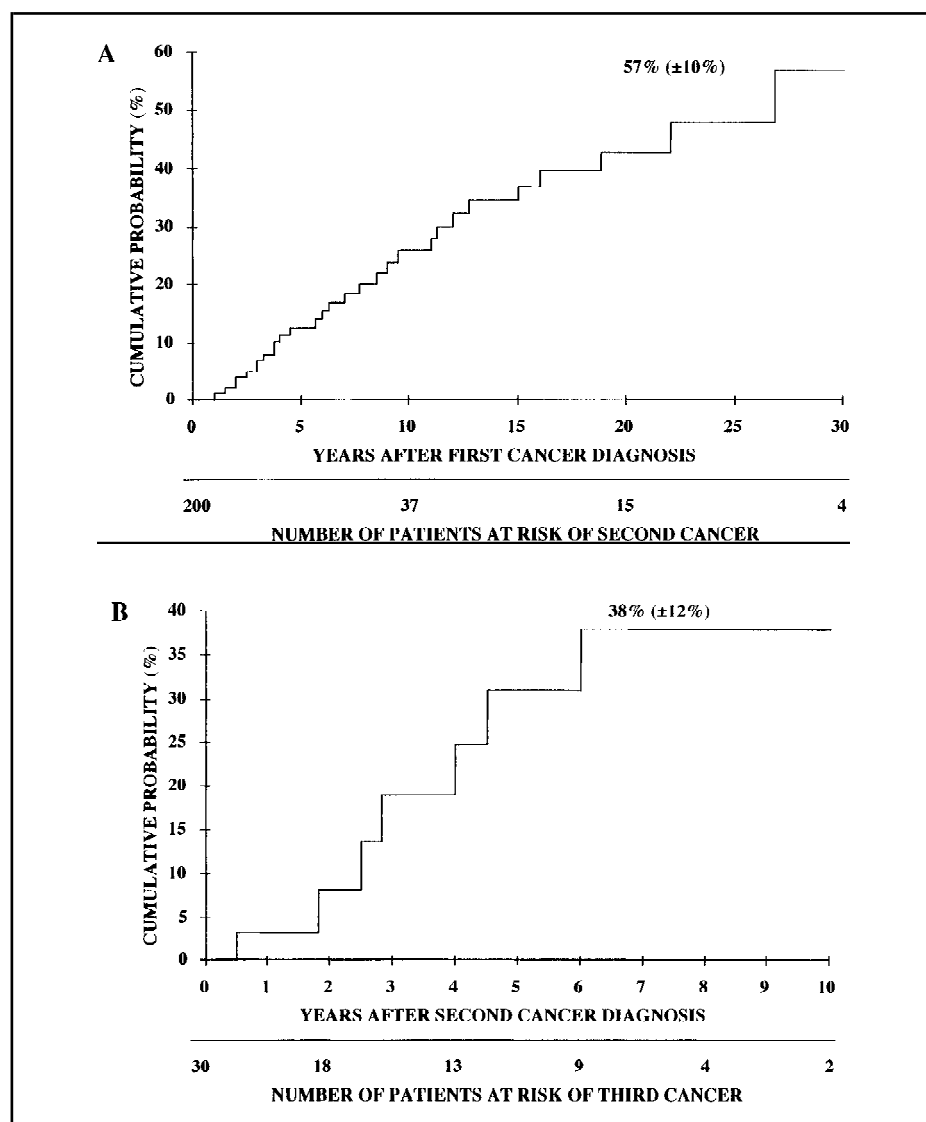


Fig. 1. A) Kaplan-Meier analysis of cumulative probability (\pm standard error) of second primary cancers during follow-up of 200 patients with a first cancer in families with Li-Fraumeni syndrome. The cumulative probability of a second cancer at 30 years was 57% ($\pm 10\%$). **B)** Kaplan-Meier analysis of cumulative probability (\pm standard error) of third primary cancers during follow-up of 30 patients with double primary cancers in families with Li-Fraumeni syndrome. The cumulative probability of a third cancer at 10 years was 38% ($\pm 12\%$).

nosis of independent primary sarcomas or breast cancers. Selective loss to follow-up is not an explanation for our findings because only two of the 200 cancer patients

in the study were lost to observation. The sharp decline in RR of second cancer with age argues against a generalized risk overestimation due to greater diligence in

Table 3. Second cancers among 200 members of Li-Fraumeni syndrome families, according to age at first cancer diagnosis*

Age at first cancer diagnosis, y	No. of study subjects	Second cancer rate/100 PY (No. of cancers/PY)	RR† (95% CI)
0-19	62	3.2 (10/312)	83.0 (36.9-187.6)
20-44	78	2.7 (14/522)	9.7 (4.9-19.2)
≥45	60	2.0 (6/308)	1.5 (0.5-4.2)
All ages	200	2.6 (30/1142)	5.3 (2.8-7.8)

*PY = person-years of observation; RR = relative risk; CI = confidence interval.

†RR was calculated by use of observed/expected number of cases: 10/0.12 for ages 0-19 years, 14/1.45 for ages 20-44 years, 6/4.08 for ages ≥45 years, and 30/5.67 for all ages combined.

seeking cancer in our families. Use of the population-based comparison data from the Connecticut Tumor Registry for all primary cancers is a standard approach to minimize the problem of inaccuracy of second cancer diagnosis and unstable estimates due to infrequent occurrence of second cancers in the general population (39,41,46). Evidence of Poisson overdispersion (Dean Score test, one-sided $P = .03$) was taken into account by the adjustment of the 95% CIs by use of Pearson's χ^2 test statistics. This approach broadened the 95% CIs by approximately 25% compared with the 95% CIs obtained with the use of the exact method.

Uncertainties exist regarding strategies to reduce second cancer morbidity and mortality in LFS families (47). The second cancers in these families can arise over several decades in diverse organs and anatomic sites, regardless of the first tumor type or the family's germline p53 gene status. Although the efficacy of screening for carriers of a mutated p53 gene is unknown, mammography and clinical breast examinations starting in early adulthood are consistent with current management strategies for carriers of the BRCA1 and BRCA2 mutations (47-49). Prophylactic mastectomy is problematic for women who have germline p53 mutations and a predisposition for additional cancer (50). Surveillance of blood cell counts to detect early leukemia can be considered, although the likelihood of finding occult leukemia is small and survival benefits are uncertain. Thus, no recommendations can be made for implementing other costly or invasive screening tests for the diverse solid tumors featured in LFS (51). It is prudent to suggest that all family members pursue a healthy lifestyle and avoid environmental carcinogens and that their physicians be alert for early signs of cancer (47). Emerging evidence for the efficacy of certain chemopreventive agents may prompt studies on genetically susceptible populations such as LFS families (47,52). In particular, chemoprevention data on p53 knockout mice can help identify candidate agents for human studies (53). However, the rarity of LFS families would necessitate a major international collaborative effort to launch a clinical trial (50).

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Notes

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Oral Transmucosal Fentanyl Citrate: Randomized, Double-Blinded, Placebo-Controlled Trial for Treatment of Breakthrough Pain in Cancer Patients

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Background: Patients with cancer frequently experience episodes of acute pain, i.e., breakthrough pain, superimposed on their chronic pain. Breakthrough pain is usually treated with short-acting oral opioids, most of which provide some relief after 15–20 minutes, with peak effects after 30–45 minutes. Oral transmucosal fentanyl citrate (OTFC), a unique formulation of the opioid fentanyl, has been shown to provide meaningful pain relief within 5 minutes in patients following surgery. We conducted a multicenter, randomized, double-blinded, placebo-controlled trial of OTFC for cancer-related breakthrough pain. **Methods:** Patients who were 18 years of age or older, receiving the equivalent of at least 60 mg oral morphine or at least 50 µg transdermal fentanyl per day for chronic cancer-related pain, and experiencing at least one episode of breakthrough pain per day were studied. After titration to an effective OTFC dose, subjects were given 10 randomly ordered treatment units (seven OTFC units and three placebo units) in the form of identical lozenges. If acceptable pain relief was not achieved within 30 minutes, subjects were instructed to take their previous breakthrough pain medication (i.e., rescue medication). Pain intensity, pain relief, and use of rescue medication were evaluated at 15-minute intervals over a 60-minute period. **Results:** Eighty-nine of 92 patients who received the randomized treatment were assessable (i.e., treated with at least one unit of OTFC and one unit of placebo). OTFC produced significantly larger changes in pain intensity and better pain relief than placebo

at all time points (two-sided $P < .0001$). Episodes treated with placebo required the use of rescue medication more often than episodes treated with OTFC (34% versus 15%; relative risk = 2.27; 95% confidence interval = 1.51–3.26; two-sided $P < .0001$). **Conclusions:** OTFC appears effective in the treatment of cancer-related breakthrough pain. [*J Natl Cancer Inst* 1998;90:611–6]

In addition to persistent pain (1), patients with cancer frequently experience superimposed intermittent episodes of acute pain, which is commonly referred to as incident or breakthrough pain (2). These transient and often intense flares of pain can be a particularly troublesome feature of chronic cancer-related pain (3). Although few studies (2,4) have been conducted to examine this problem specifically, recent reports indicate that breakthrough cancer pain, severe to excruciating in intensity, occurs in up to 65% of patients with cancer and is frequently undertreated.

The current standard of care for treating cancer pain is to provide a sustained-release preparation that controls the chronic, persistent pain and a rapid, relatively short-acting analgesic that relieves the breakthrough pain without lingering so long as to cause somnolence once the painful episode has subsided. Although data demonstrating efficacy have not been published, the mainstays of breakthrough pain therapy are short-acting oral opioids that are generally believed to have an onset of 15–20 minutes and a peak effect after 30–45 minutes.

Oral transmucosal fentanyl citrate (OTFC) is a unique formulation in which fentanyl, a potent and short-acting opioid that binds primarily to the morphine (mu)

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receptor, is incorporated into a sweetened lozenge attached to a stick. The fentanyl is absorbed through the oral mucosa as the lozenge dissolves in the mouth (5). OTFC has been shown to have properties of onset and peak activity similar to those of intravenous morphine (6). Of the total available dose, 25% is absorbed transmucosally over a 15-minute period, and an additional 25% is absorbed through the gastric mucosa during the next 90 minutes (7). The onset of meaningful relief has been shown to occur as quickly as 5 minutes in patients with postoperative pain (6). The pharmacokinetics of OTFC in patients with cancer were evaluated in another study (8).

OTFC has been useful for the management of breakthrough pain in patients with cancer in two open-label reports (9,10).

In this report, we present data from a multicenter, randomized, placebo-controlled, double-blinded clinical trial to determine whether patients with breakthrough cancer pain obtain clinically important pain control more often with the active fentanyl product than with an identical placebo delivery system alone.

Patients and Methods

Patients with cancer who had relatively stable pain and who were 18 years of age or older were recruited from 23 different community and academic cancer centers (see "Appendix: Study Group List"). This study was approved by the institutional review board at each study site, and all patients gave written, informed consent prior to participation. Most patients were known to the investigators, but a few were referred by the local physicians' network specifically for this trial. All types and stages of cancer were acceptable, provided the patients reported sufficient pain to require at least the equivalent of 60 mg/day oral morphine or at least 50 μ g/hour transdermal fentanyl and had at least one episode of breakthrough pain per day for which they took additional opioids. Patients were provided with free study medication but were not otherwise compensated for their participation.

A thorough medical history was recorded, and a physical examination was carried out to collect demographic data and to ensure that there was no history of psychiatric disease or of drug abuse as well as no evidence of oral, hepatic, renal, or cognitive disease that would prevent participation in the study. All study subjects were started on 200 μ g OTFC (developed by Anesta Corp., Salt Lake City, UT, and distributed by Abbott Laboratories, Abbott Park, IL) as a replacement for their prescribed breakthrough medication as part of an open-label dose titration. Subjects were taught how to consume the total dose within 15 minutes and were instructed that, if they did not perceive adequate pain relief

after 30 minutes, they were allowed to take a dose of their usual rescue medication (i.e., their previous breakthrough pain medication). For each episode of breakthrough pain treated with an OTFC unit, all subjects were taught how to fill out the medication diary, including the time, the date, and product information (i.e., placing the peel-off sticker from the individual dose package into the diary). The validated pain scales (11,12) used in this study included pain intensity (0 = no pain \rightarrow 10 = worst pain), pain relief (0 = none \rightarrow 4 = complete), and global performance evaluation (0 = poor \rightarrow 4 = excellent). Information on whether the patient decided to take additional medication for the relief of pain for each episode (yes or no) was also collected as a novel outcome with clear clinical importance. Since previous studies on dosing (5,8,9) were not able to define a consistent analgesic-equivalency table for conversion of other opioid rescue medications to the appropriate OTFC dose, all subjects were started on the lowest dose (200 μ g) and maintained close contact with study staff to ensure a safe titration. They were then titrated to an effective dose up to the maximum available dose (1600 μ g) over a 2-week period. An effective dose was defined as the dose required to treat most episodes of breakthrough pain with a single OTFC unit. Subjects were instructed to return their diaries, used OTFC containers, and unused doses at each clinic visit. The diaries were reviewed by a research nurse in the presence of the subject to ensure accurate and complete data entry.

All subjects who were able to achieve adequate relief with OTFC were advanced to the randomized, double-blinded phase, which was designed as a 10-period crossover. In this phase, each subject was given a box of 10 sequentially numbered units. Of the 10 units, seven contained fentanyl at the same dose found effective for that patient in the titration phase, and three were placebo units. So that we could maintain study blinding, the placebo doses were formulated identically (i.e., color, taste, and texture) and packaged identically to the active drug. The ordering of the placebos and active units was random for each patient, with one placebo in the first three units, another in the second three units, and one in the last four units, but always with a separation of at least one active dose between two placebos (for ethical reasons). A sealed key was provided with each study box for emergency use, but none was needed during the study. One third of the patients had placebo as a first dose, one third had it as a second dose, and one third had it as a third dose. Of the 804 episodes of pain treated, 247 (30.7%) were treated with placebo and 557 (69.3%) were treated with active drug.

Subjects were instructed to use the units in sequential order, with a minimum of 2 hours between episodes treated with OTFC, and to record the unit number for each one used by placing the peel-off sticker from the unit in the appropriate box in their study diary. If pain relief was not adequate within 30 minutes, patients were encouraged to take a dose of their previous non-study breakthrough pain medication. *A priori* criteria were established to deal with protocol violations, including an interval of less than 2 hours between doses of OTFC, variation of more than 10 minutes in any of the four required 15-minute recordings following the consumption of a unit (i.e., at 15, 30, 45, and 60 minutes), incomplete consumption of a unit, treatment of a pain different

from that originally designated, and incomplete records. These rules were applied to each treated episode before the blinding was broken. For the primary analysis (but not for the intention-to-treat analysis), protocol violations were excluded. After completing the randomized phase, all patients were given the option to continue the use of OTFC for as long as they found the product effective for their breakthrough pain.

The original protocol specified the primary outcome as the sum of the pain intensity differences (i.e., the area under the curve of the pain intensity differences) and the total pain relief (i.e., the area under the curve of the pain relief values), calculated after the exclusion of all episodes found to have significant protocol violations (74 episodes). However, since both of these measures require imputation of data, an intention-to-treat analysis is presented first and includes all data from all patients who took at least one active and one placebo dose (801 episodes). The average pain intensity difference and pain relief are reported at each time point. Since individuals were allowed to take an additional dose of their previous medication after 30 minutes, not all subjects had 45-minute and 60-minute values. The total number of assessable subjects at each time point is presented in the "Results" section. To calculate the sum of the pain intensity differences and the total pain relief, we used the conservative last occurrence carry forward method to impute missing values for the 45-minute and 60-minute time periods in subjects who decided to take additional rescue medication before the full 60-minute recording period had elapsed. The sum of the pain intensity differences is calculated by subtracting the pain intensity at any point from the baseline (i.e., 0 minutes) and cumulatively adding up these values over the four measurement times of the study (i.e., 15, 30, 45, and 60 minutes) (13). The total pain relief is calculated by cumulatively adding the pain relief measured at each time period (13).

The mean values of the episodes treated with active drug and the episodes treated with placebo were assessed for each time period by use of a paired *t* test. Since each patient had multiple exposures to both placebo and active drug, generalized evaluation equations were used to account for the lack of independence of the episode data by clustering the episode values for each subject to provide an accurate *P* value for clustered data (14,15). As noted above, all patients who consumed at least one active and one placebo unit were included in the analysis. The same method was used to perform a secondary analysis of the subjects' reported satisfaction with the treatment and whether they took additional rescue medication. Baseline subject characteristics and side effects are reported descriptively. All statistical analyses were performed with the use of SAS (version 6.01; SAS Institute, Cary, NC) and STATA (version 5.p; STATA Corporation, College Station, TX) software. All reported *P* values are two-sided.

Results

Of the 130 patients originally recruited, 93 completed the open-label titration phase and 37 did not. The primary reasons for not completing the open-label

phase were patient choice ($n = 15$), advancing cancer limiting the patient's ability to take the drug ($n = 12$), and specific side effects ($n = 10$). The specific side effects were nausea/vomiting ($n = 6$), mental status changes ($n = 2$), and dyspnea ($n = 2$). Of the two patients withdrawn for dyspnea, reported as possibly related to the OTFC, one had three mild episodes associated with anxiety in addition to a known history of anxiety-related dyspnea, and the other had lung cancer, chronic obstructive pulmonary disease, and pulmonary emboli. Of the 15 who chose not to continue, four reported that their breakthrough pain spontaneously ceased or substantially decreased, four preferred their previous medication, four were not able to complete the diaries successfully, one was lost to follow-up, and two did not specify a reason.

Of the 93 patients who achieved adequate pain relief with OTFC and were eligible for the randomized, double-blinded phase, 92 agreed to participate. Three of these patients took only one unit (placebo, $n = 2$; OTFC, $n = 1$) before dropping out, which left 89 patients in the intention-to-treat analysis. In all, 20 patients did not complete the full 10 doses of the double-blinded phase. Eight of these 20 patients completed four doses or fewer, six completed five doses, four completed six doses, and two completed seven doses, with the remaining 72 patients completing all 10 doses. Of those not completing all 10 doses, 10 did not complete the randomized phase in the required 14 days, six had progression of their cancer, two developed nausea/vomiting or itching, and two chose to discontinue for unspecified reasons. Given the crossover design, all patients served as their own controls.

Table 1 displays demographic data for all 92 patients who chose to participate in the randomized, double-blinded phase, with the primary cancer diagnosis and the type of pain indicated. Overall, there was no statistically significant difference in any demographic variable or type of tumor between those who completed the randomized phase and those who did not. In addition, the majority of the patients were taking oral morphine (68% [$n = 63$]; dose range, 30–600 mg per day) or using the fentanyl patch (23% [$n = 21$]; dose range, 50–225 μg per hour) as their around-the-clock medication. The rescue

Table 1. Characteristics of all patients who participated in the randomized, double-blinded phase of the trial of oral transmucosal fentanyl citrate for cancer-related breakthrough pain

Variable*	Completed double-blinded phase	Did not complete double-blinded phase	Total
No. of patients	72	20	92
Sex			
No. of females (%)	39 (54)	12 (60)	51 (55)
No. of males (%)	33 (46)	8 (40)	41 (45)
Age, y			
Mean \pm SD	53 \pm 11	57 \pm 15	54 \pm 12
Range	27–77	29–84	27–84
Height, cm			
Mean \pm SD	169 \pm 10	167 \pm 11	169 \pm 10
Range	150–193	142–188	142–193
Weight, kg			
Mean \pm SD	71 \pm 21	66 \pm 13	70 \pm 20
Range	42–128	40–91	40–129
Race			
No. black (%)	3 (4)	2 (10)	5 (5)
No. Asian (%)	1 (1)	0 (0)	1 (1)
No. white (%)	68 (94)	18 (90)	86 (93)
Cancer type			
No. breast (%)	18 (25)	3 (15)	21 (23)
No. lung (%)	14 (19)	3 (15)	17 (18)
No. colon/rectal (%)	11 (15)	1 (5)	12 (13)
No. uterine (%)	6 (8)	1 (5)	7 (8)
No. other—solid tumor (%)	14 (19)	9 (45)	23 (25)
No. other—hematologic (%)	9 (13)	3 (15)	12 (13)
Pain type			
No. somatic (%)	38 (53)	10 (50)	48 (52)
No. visceral (%)	22 (31)	7 (35)	29 (32)
No. neuropathic (%)	11 (15)	2 (10)	13 (14)
No. unknown (%)	1 (1)	1 (5)	2 (2)

*SD = standard deviation.

medication replaced by OTFC (and the percentage of patients affected) included oxycodone (37%), morphine (30%), hydrocodone (13%), hydromorphone (12%), and other medications (8%).

The primary comparison of the pain intensity differences and pain relief in an intention-to-treat analysis is shown in Fig. 1. A comparison of the primary outcome analyses for pain intensity differences and pain relief, excluding patients with protocol violations, is shown in Fig. 2. Eighty-six patients were included in the latter efficacy comparisons; six patients were not included because of protocol violations. The 86 patients generated assessable data from 730 episodes of pain. For all time periods, statistically significant differences ($P < .0001$) were seen between episodes treated with OTFC and episodes treated with placebo. The mean global performance evaluation scales were 1.98 for OTFC and 1.19 for placebo ($P < .0001$). In addition, subjects required significantly more additional rescue medication for breakthrough pain episodes treated with placebo than for epi-

sodes treated with the active drug (34% versus 15%; relative risk = 2.27; 95% confidence interval = 1.51–3.26; $P < .0001$). Specifically, patients using placebo were more than twice as likely to require an additional rescue dose as were those who used the active agent. Of the original 92 patients, 74 chose to continue to treat their breakthrough pain with OTFC following the randomized clinical trial. No specific subgroup could be identified that was more or less responsive to OTFC.

Table 2 lists the primary opioid-related adverse events reported for all 130 patients initially enrolled in the trial. Most of the adverse events that occurred in the study were reported by the site investigator as likely due to other treatments or to the cancer itself, as would be expected in patients with cancer. The more frequent opioid-related adverse events reported as possibly related to OTFC were dizziness (17%), nausea (14%), somnolence (8%), constipation (5%), asthenia (5%), confusion (4%), vomiting (3%), and pruritus (3%).

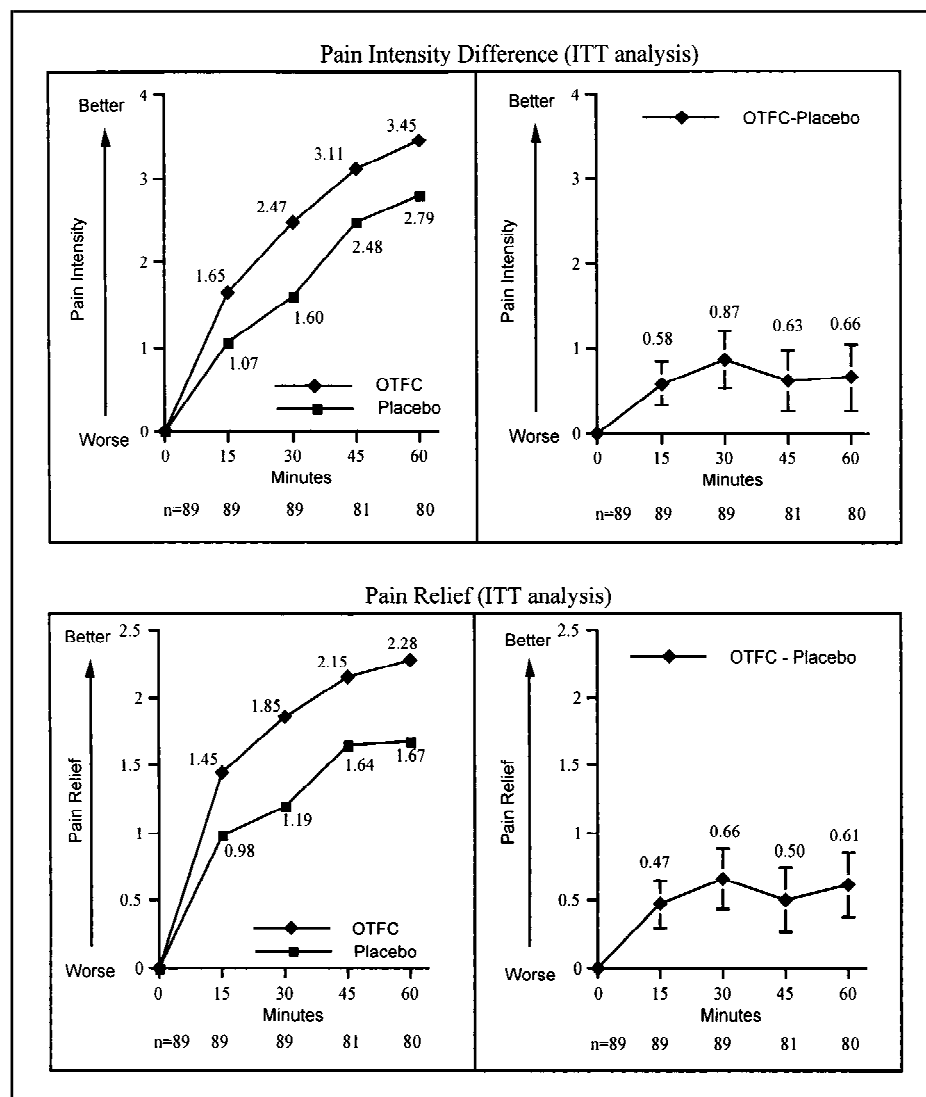


Fig. 1. Intention-to-treat (ITT) analysis of pain intensity differences and pain relief. All patients who entered the double-blinded phase of the trial and who received both oral transmucosal fentanyl citrate (OTFC) and placebo were included. Data were not available for all patients at all time periods. Ninety-five percent confidence intervals are shown for the OTFC minus placebo (i.e., OTFC - Placebo) values for this paired analysis. See text for additional details.

Discussion

Pain remains a substantial problem for most patients with cancer. Although the primary impediment to good care worldwide is the inadequate use of currently available pain medications, one of the more difficult aspects of pain treatment has been breakthrough or incident-related pain (1). Even in patients with well-controlled chronic components to their pain, the intermittent pain associated with daily activity or movement can be disabling. This type of pain usually begins relatively acutely and can be quite severe, especially in patients with musculoskeletal metastasis. In one 3-month survey (2) of 63 patients with cancer, 41 (65%) reported one or more episodes per day of

transient flares of severe or excruciating pain with an overall duration of 1–240 minutes and a median duration of 30 minutes. The pathophysiology of the pain was attributed approximately equally to somatic, neuropathic, visceral, and mixed causes, although the accuracy of such diagnoses is hard to determine (2). The limited literature that exists suggests that the best treatment should consist of a fast-acting drug that has a relatively short half-life, so that the effects of the medication resolve as the pain abates. The usual dose for each episode of pain is 10%–15% of the total 24-hour around-the-clock dose taken at the onset of pain or just before predictable episodes, such as moving a patient with a broken bone. To date, we

have been limited to oral (convenient but relatively slow), rectal (relatively slow and inconvenient for frequent use), or parenteral (more rapid but inconvenient and costly) treatments. The transmucosal route is convenient and has a rapid onset, representing an important addition to the potential therapeutic options.

There are several important aspects of this study. The first is that OTFC was found to be statistically significantly better than the placebo in every analysis completed, looking at the changes in the mean values of pain intensity, pain relief, and global performance as well as in the proportion of pain episodes for which subjects required an additional rescue medication (i.e., clinically important change). Therefore, this new delivery system is highly effective in treating episodes of breakthrough pain in patients with cancer. Our study did not show that any specific cancer type or disease pattern was more or less susceptible, but the study was not powered for subgroup analyses; thus, the final answer to this question remains to be resolved.

Second, when properly used in patients who are tolerant to opioids, OTFC has relatively few important side effects. Despite the relatively high doses of fentanyl used, there were no serious events, such as respiratory depression or severe somnolence, attributed to OTFC. However, the dose of fentanyl citrate is large enough that there may be concern about respiratory depression in the opioid-naïve patient.

Third, although patients on higher doses of original rescue medication generally required larger doses of OTFC, this relationship was not consistent enough to determine a reliable equivalency ratio, perhaps because rapid absorption changes the pharmacodynamics of treatment.

Fourth, the trial incorporated a number of design advantages and features that were developed specifically for this study. This unique combination of features can be applied to future trials of medications that have rapid onset and potential efficacy in the treatment of breakthrough or acute pain. Specifically, the titration run-in period clearly defines a potentially responsive group of patients, while it also provides invaluable information about patients who may not benefit as much from the therapy. The use of a group of randomly ordered active and placebo medi-

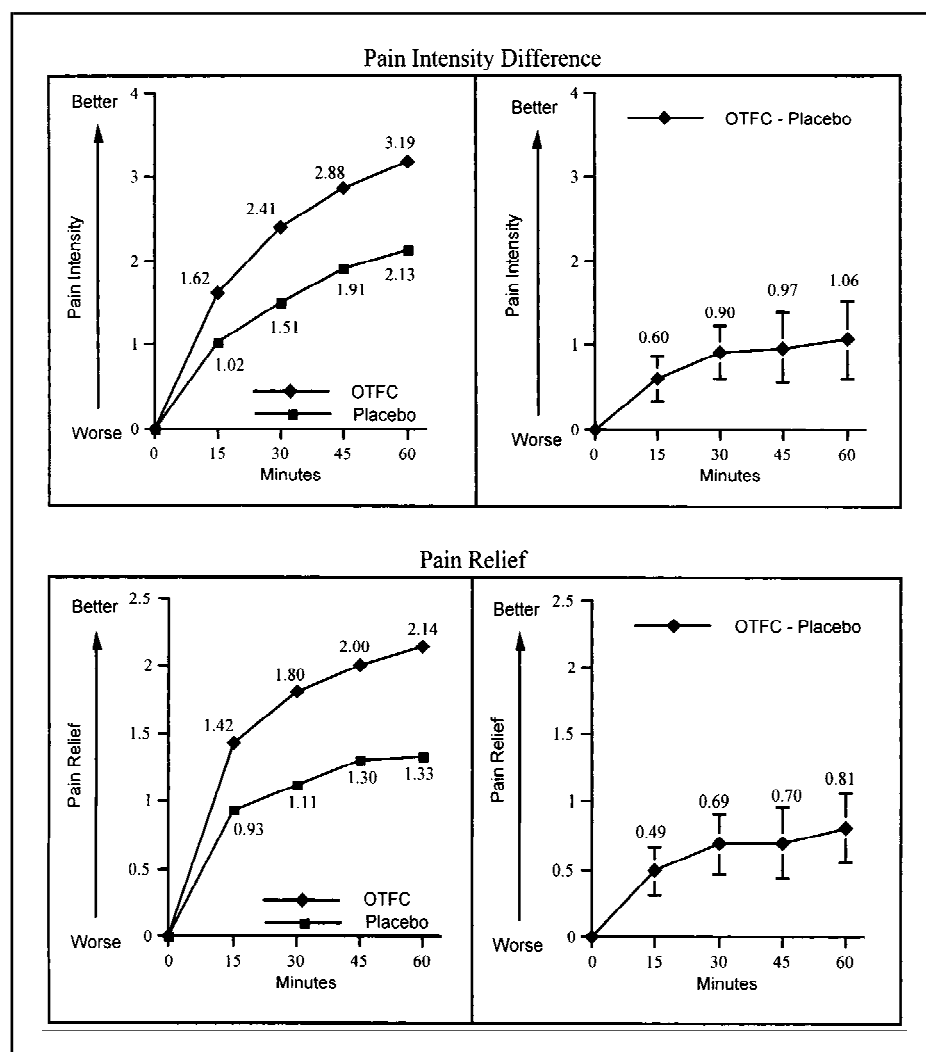


Fig. 2. Primary outcome analyses for pain intensity differences and pain relief. After exclusion of individuals with procedural violations and use of the last occurrence carry forward method to input missing values, data from 86 patients who entered the double-blinded phase of the trial and received both oral transmucosal fentanyl citrate (OTFC) and placebo were included in the analyses. Ninety-five percent confidence intervals are shown for the OTFC minus placebo (i.e., OTFC - Placebo) values for this paired analysis.

Table 2. Primary opioid-related adverse events for all 130 patients initially enrolled in the trial of oral transmucosal fentanyl citrate (OTFC) for cancer-related breakthrough pain

Typical adverse events*	No. of patients (%)
Dizziness	22 (17)
Nausea	18 (14)
Somnolence	11 (8)
Constipation	7 (5)
Asthenia	6 (5)
Confusion	5 (4)
Vomiting	4 (3)
Pruritus	4 (3)

*Only adverse events that were considered by the investigator to be at least possibly related to the study drug and that occurred on days when an OTFC unit was used are included.

cations for the trial portion of the study, along with a short waiting period before the use of additional rescue medication if needed, provides an ethical way to incorporate placebo controls into an efficacy trial. Given the significant advantages of a placebo control group and the clear ethical issues surrounding the administration of a placebo to sick patients, this feature is important. The frequent measurement of several pain-related scales (especially the measure of those who required additional rescue medications for individual episodes of breakthrough pain) adds additional validity to the results. This measure is clearly different from the more standard time-to-next-rescue response, which incorporates both initial activity and length

of effect in a way that can introduce a level of ambiguity into the analysis.

Fifth, some of the subgroup analyses provide interesting hypotheses for future consideration and confirmation. One is that, of the 92 subjects in the randomized phase of the trial, 13 (14%) were reported to have a substantial component of neuropathic pain, which is usually considered to be only partially responsive to opioid therapy (16). Despite this fact, 11 (85%) of the 13 reported clinically important relief with OTFC in the first phase of the study. This result emphasizes the considerable variation in our ability to diagnose and treat different types of pain and is consistent with the idea that most cancer patients have a mixed pain syndrome (16). Our finding suggests that we should not withhold OTFC therapy simply because a patient is thought to have a predominantly neuropathic pain syndrome.

Sixth, it is interesting that 66% of the episodes treated with placebo did not require an additional dose of medication, which is in the upper range for reported placebo responses (17). However, this rate is completely consistent with the disease process, the type of pain, the patient population, and study design in this trial. It is likely that a large portion of this phenomenon can be explained by the normal course of episodes of breakthrough pain, which are often relatively short-lived and improve spontaneously over a time course similar to that which subjects expect when taking the active drug. An additional portion of these episodes might be explained by a true placebo response in which endogenous opioid production or a neurologic down-regulation response (i.e., pain suppression) makes an important contribution to the improvement of the patient's pain.

The limitations of this study are primarily those common to any randomized clinical trial. Since only patients with cancer and clearly defined breakthrough pain treated with chronic opioids were recruited, the generalization of these results to other populations should be done in a carefully considered manner. This medication had a high degree of safety in this closely monitored and opioid-tolerant population; however, the potential for side effects with inappropriate use implies that considerable caution be used in initiating therapy, especially for patients who are opioid naive. In addition, of the 130

patients initially selected to use this medication, 56 (43%) ultimately did not continue to use OTFC beyond the clinical trial period. This value is consistent with other dosage forms and types of opioids (18) used to treat cancer-related pain. A majority of these patients developed problems related to important progression of their disease. However, since patients with cancer are the primary target population for this treatment, careful consideration must be given to those who can benefit the most from this form of therapy. The clear advantages of rapid onset and relatively short duration of action may make this form of medication delivery less appropriate for patients whose breakthrough pain is of longer duration.

In conclusion, the OTFC drug-delivery system described here is a highly efficacious treatment for cancer-related breakthrough pain and shows a large margin of safety in patients on chronic opioid therapy. In view of our results and other published findings (9), the advantages of rapid onset, transmucosal absorption (i.e., no need to swallow), titrateability, ease of use, and acceptance by patients make OTFC ideally suited for this purpose.

Appendix: Study Group List

We would like acknowledge the other members of the Anesta Management of Pain Symptoms (AMPS) study group for their collaborative efforts.

The AMPS study group included the following: Robert Berris, M.D., Rocky Mountain Cancer Centers, Denver, CO; Allen Cohn, M.D., University of Colorado Health Sciences Center, Denver; Robert Ellis, D.O., Madigan Army Medical Center, Tacoma, WA; Janet Gargiulo, M.D., Capital District Hematology/Oncology Associates, Latham, NY; Stuart Grossman, M.D., The Johns Hopkins Oncology Center, Baltimore, MD; Lowell Hart, M.D., Associates in Hematology and Oncology, Fort Meyers, FL; Laurel Herbst, M.D., San Diego Hospice, CA; Howard Homesley, M.D., North Carolina

Baptist Hospital/Carolina Gynecologic Oncology, Winston-Salem; Laura Hutchins, M.D., Arkansas Cancer Research Center, Little Rock; K. S. Kumar, M.D., United Professional Center, New Port Richey, FL; Michael Levy, M.D., Fox Chase Cancer Center, Philadelphia, PA; John Marshall, M.D., Vincent T. Lombardi Cancer Center, Washington, DC; Timothy J. Ness, M.D., University of Alabama—Birmingham; Kelly Pendergrass, M.D., Kansas City Internal Medicine, MO; Lee Schwartzberg, M.D., The West Clinic, Memphis, TN; Mark Seligman, M.D., Providence Hospice, Portland, OR; Gregory B. Smith, M.D., SW Regional Cancer Center, Austin, TX; Charles von Gunten, M.D., Northwestern University, Chicago, IL; William H. Whaley, M.D., West Paces Medical Center, Atlanta, GA; Donna Saltzburg Zhukovsky, M.D., The Cleveland Clinic Foundation, OH.

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Notes

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Human Leukocyte Antigen Class II Alleles Associated With Human T-Cell Lymphotropic Virus Type I Infection and Adult T-Cell Leukemia/Lymphoma in a Black Population

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Background: Human T-cell lymphotropic virus type I (HTLV-I) is linked to adult T-cell leukemia/lymphoma (ATL) and HTLV-I-associated myelopathy (HAM; also known as tropical spastic paraparesis [TSP]), a chronic neurodegenerative disorder. Worldwide, several million HTLV-I carriers are at risk for disease, with an estimated lifetime cumulative risk of 1%–5%. However, the determinants of disease progression are relatively unknown. We studied human leukocyte antigens (HLA class II) that have been implicated in the pathogenesis of HTLV-I-related diseases. **Methods:** We analyzed HLA class II alleles among asymptomatic HTLV-I carriers (n = 45), patients with ATL (n = 49) or HAM/TSP (n = 54), and HTLV-I seronegative control subjects (n = 51). All participants were of African descent and were enrolled in epidemiologic studies conducted at the University of the West Indies, Kingston, Jamaica. We used standard microlymphocytotoxicity assays for HLA antigen serotyping and polymerase chain reaction-based methods to examine HLA class II DRB1 and DQB1 alleles. **Results:** Two antigens determined by serotyping, DR15 and DQ1, occurred at significantly increased frequency among HTLV-I carriers compared with seronegative control subjects (42% versus 22% for DR15 [odds ratio {OR} = 2.7; 95% confidence interval {CI} = 1.0–

7.2] and 78% versus 53% for DQ1 [OR = 3.1; 95% CI = 1.2–8.5]). Asymptomatic carriers were shown to have an HLA class II allele distribution similar to that of patients with ATL, and the frequencies of the alleles DRB1*1501, DRB1*1101, and DQB1*0602 were significantly greater in patients with ATL and asymptomatic carriers than in patients with HAM/TSP. In addition, haplotypes DRB1*1101-DQB1*0301 and DRB1*1501-DQB1*0602 were significantly increased among patients with ATL compared with patients with HAM/TSP. **Conclusions:** These data suggest that host genetic background is an important factor in determining whether HTLV-I carriers develop either ATL or HAM/TSP. [J Natl Cancer Inst 1998;90:617–22]

Human T-cell lymphotropic virus type I (HTLV-I) infection is associated with an increasing spectrum of diseases (1,2) but is most definitively associated with adult T-cell leukemia/lymphoma (ATL), a rapidly, fatal T-cell lymphoma (3), and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), a chronic neurodegenerative disorder (4). HTLV-I is endemic in southern Japan; the Caribbean; parts of South America, Africa, and the Middle East; and the Melanesian Islands. The virus has also been identified in areas within the United States and other parts of North America and Europe, particularly among immigrant populations from endemic areas (5). HTLV-I-related diseases cluster in areas that are endemic for the virus. The annualized incidence of ATL and HAM/TSP, among major endemic populations, has been estimated as 16 and 22 cases in 100 000 HTLV-I carriers, respectively (6,7). It has been hypothesized that ATL develops after a long latent period of approximately 20–40 years following childhood infection primarily due to maternal transmission of HTLV-I via breast-feeding (3). HAM/TSP has been attributed predominantly to sexually or transfusion-acquired infection, with disease developing in days to years following infection (8,9). Determinants of the pathogenesis of these distinct disease entities are not clearly understood. However, the differential immune response to the virus among HTLV-I in-

fected, asymptomatic carriers (AC) has been implicated.

Major histocompatibility complex (MHC), human leukocyte antigens (HLAs) class I (A, B, and C loci), and class II (DR and DQ loci) genes, located on chromosome 6, have a unique role in regulating the immune response to infection and malignant transformation (10, 11). Experiments among inbred mice pioneered in the mid-1960s were among the first to show possible genetic control of the immune response by the MHC (12). These studies recognized that specific MHC loci were linked to ability to mount an antibody response. Similar to this animal model, a human corollary is represented by the differential antibody response among patients with HTLV-I-related disease, with patients with HAM/TSP having elevated antibody levels compared with HTLV-I carriers and patients with ATL (13). Additionally, Usuku et al. (14) have reported that host immunogenetic background, reflected by level of spontaneous lymphocyte proliferation, can be linked to HLA haplotypes that differ between patients with ATL and patients with HAM/TSP. HLA haplotypes associated with ATL are characterized by a low spontaneous lymphocyte proliferation response, while those of HAM/TSP represent high responders (15). It is likely that similar host factors are important in other HTLV-I endemic populations, as suggested by preliminary studies conducted in Jamaica that reveal possible disease associations with particular HLA class II alleles (16).

To confirm the role for HLA class II as an important host-related immunogenetic factor, we have examined in this report whether HLA class II alleles are associated with HTLV-I infection and its related

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disease outcomes, ATL and HAM/TSP, in a homogeneous Jamaican population of African descent.

Methods

Study population. Sera and lymphocyte specimens were collected from a population of healthy workers employed in the food handler industry in Jamaica. This population was previously described in a survey of the island-wide seroprevalence of HTLV-I in Jamaica conducted from 1984 through 1985 (17). Among participants from the previous study, all HTLV-I seropositive and HTLV-I seronegative individuals from two parishes, Kingston and Clarendon, were re-visited from 1987 through 1988 and peripheral blood lymphocyte samples were obtained. We initially selected 100 food handlers for the current analysis. The participants chosen were of African descent with known HTLV-I status, who were otherwise designated without regard to other factors. Two individuals were subsequently deleted because their race was not correctly classified. Two additional samples were deleted because of insufficient DNA for typing. Final analysis was reported for 96 of the 100 individuals, 45 who were HTLV-I seropositive (47%) and 51 who were HTLV-I seronegative (53%). Sera and lymphocyte specimens were also evaluated from unrelated subjects with clinically and/or pathologically characterized ATL ($n = 49$) and HAM/TSP ($n = 54$), who were enrolled in case registries maintained at the University of the West Indies, Kingston, Jamaica. All patients were participants in epidemiologic studies (3,9,18) approved by the Human Subjects Review Committees at the University of the West Indies and the National Cancer Institute, Bethesda, MD. All individuals with ATL and HAM/TSP were HTLV-I seropositive.

Laboratory procedures. HTLV-I serologic status was determined in sera by enzyme-linked immunosorbent assay (ELISA; E. I. Du Pont de Nemours Company, Wilmington, DE, or Genetic Systems, Seattle, WA) and confirmed with western blot (Cambridge-Biotech, Rockville, MD). Antibody titers were determined for all HTLV-I seropositive carrier samples by end-point-dilution ELISA testing with fourfold dilutions. Heparinized whole blood samples were obtained and peripheral blood lymphocytes were separated by Ficoll-Hypaque density gradient centrifugation at 2100 rpm/900g (19). Peripheral blood lymphocytes were stored at -70°C in a liquid nitrogen freezer until used. Serologic typing for HLA was performed on HTLV-I carriers and seronegative control subjects using the cryopreserved cells by the standard antibody-mediated National Institutes of Health microlymphocytotoxicity test (20) with qualified HLA trays containing 213 antisera for different HLA-A, -B, and -C antigens and 70 antisera for different HLA-DR and -DQ antigens. All sera were exchanged with reference laboratories and standardized to the assignment of the 11th (1991) International Histocompatibility Workshop (21). DNA typing for HLA class II alleles (DRB1 and DQB1) was performed on all HTLV-I carriers, ATL and HAM/TSP cases by polymerase chain reaction (PCR) on DNA extracted from cryopreserved cells using PCR sequence-specific oligonucleotides (ELPHA-Biotest; Biotest Diagnostics Corp., Dreieich, Germany) or PCR restriction-fragment-length poly-

morphisms (SMITEST; Sumitomo Metal Industries, Ltd., Tokyo, Japan). All HLA typing (serology, microcytotoxicity, and PCR assays) was performed in the laboratory of S. Sonoda at Kagoshima University in Japan.

Statistical analysis. HLA data were analyzed to evaluate a possible association of class II alleles with HTLV-I infection, ATL, or HAM/TSP. HLA class II antigen (DR and DQ) and allele (DRB1 and DQB1) frequencies were calculated and comparisons were made with chi-squared (likelihood ratio [G] test) (22) and Fisher's exact test (23) to detect an association of a specific allele with HTLV-I infection or related disease, using Statistical Analysis Software Program (SAS Institute, Cary, NC). All P values reported were two-tailed, with significance defined as $P < .05$. When case groups were found to be significantly different from control subjects, the odds ratio (OR) with 95% confidence interval (CI) associated with specific class II alleles was calculated. To eliminate the possibility that significant associations were due to chance because multiple comparisons of antigens/alleles between groups were made concurrently, the data were adjusted by the omnibus chi-squared [G] test (22). If this test was significant, it suggested a statistical difference in the frequencies at the locus tested. When this global test showed no significance, a Bonferroni's correction was performed for the individual antigens/alleles showing a significant difference (24). Additionally, we tested an *a priori* hypothesis for specific significant alleles by comparing our findings with those reported in the literature for another HTLV-I endemic population group. Linkage disequilibrium between two alleles in different loci was also evaluated (25). HTLV-I antibody geometric mean titers were calculated by taking the mean of the log of the actual titer value. The Student's t test was used to examine the significance of a difference between two means.

Results

HLA and HTLV-I Infection

The HTLV-I carrier and seronegative control groups were homogeneous with respect to sociodemographic data. The median age was 45 years in both groups. Eighty-two percent of both the HTLV-I-positive and HTLV-I-negative subjects were female, which was consistent with the larger population from which samples were drawn. The majority of study participants (89% of HTLV-I-positive and 82% of HTLV-I-negative subjects) were born outside of Kingston. Nearly every participant (100% of HTLV-I-positive and 96% of HTLV-I-negative subjects) had attended some school, although only a modest number had completed secondary education or higher (36% of HTLV-I-positive and 35% of HTLV-I-negative subjects). A slightly higher proportion of HTLV-I-positive (55%) compared with HTLV-I-negative (43%) individuals

earned income at or above the minimum wage.

Table 1 shows class II antigens with significant statistical differences between HTLV-I-positive and HTLV-I-negative subjects. Two class II antigens, DR15 and DQ1, were significantly higher among HTLV-I-positive subjects (OR = 2.7; 95% CI = 1.0–7.2 and OR = 3.1; 95% CI = 1.2–8.5, respectively). Even after applying the Bonferroni's adjustment for multiple comparisons, the elevation of the antigen DQ1 among HTLV-I-positive subjects was still significant. The increase of DR15 among HTLV-I-positive subjects was not significant by this conservative adjustment. However, these two antigens were found to be in linkage disequilibrium, with DR15 detected only in the presence of DQ1. The coefficient of disequilibrium was stronger among HTLV-I-positive (0.113) than HTLV-I-negative (0.078) subjects. DQ1 alone or in combination with DR15 was detected in 78% of HTLV-I-positive subjects compared with 53% of HTLV-I-negative subjects ($P = .01$, $P = .049$ with Bonferroni's correction).

To evaluate whether any of the antigens at higher frequency among HTLV-I

Table 1. Comparison of HLA-DR and -DQ antigen frequencies between HTLV-I-positive carriers and HTLV-I-negative control subjects

HTLV-I positive (n = 45)			HTLV-I negative (n = 51)		χ^2*
No.			No.	%	
HLA-DR serotypes					
1	9	20	8	16	0.3
3	10	22	19	37	2.6
4	2	4	3	6	0.1
7	7	16	5	10	0.7
8	15	33	15	29	0.2
9	2	4	3	6	0.1
10	1	2	1	2	0.01
11	11	24	16	31	0.6
12	4	9	3	6	0.3
13	3	7	6	12	0.7
14	2	4	5	10	1.1
15	19	42	11	22	4.8 ¹
16	0	0	1	2	1.3
52	37	82	45	88	0.7
53	10	22	8	16	0.7
HLA-DQ serotypes					
1	35	78	27	53	6.6 ²
2	11	24	13	25	0.01
3	3	7	6	12	0.8
4	10	22	18	35	2.0
7	19	42	23	45	0.1

* χ^2 = likelihood ratio chi-squared (G test). ¹ $P = .029$; ² $P = .01$ ($P = .049$ by Bonferroni's correction).

positive carriers corresponded to a differential viral immune response, we measured HTLV-I antibody titers among seropositive subjects. We observed no difference in proportion of individuals having elevated titers above the median level (1:9886) for HTLV-I carriers with DR15-DQ1 haplotype (47%), DQ1 only (56%), or other antigens (50%). The geometric mean titer (GMT) was also calculated for these same groups and they did not differ. GMT among HTLV-I carriers was 1:12 599 for those with DR15-DQ1, 1:10 355 for those with DQ1 only, and 1:11 119 for HTLV-I carriers with other HLA-class II antigens.

HLA and HTLV-I-Associated Disease: ATL and HAM/TSP

Because HLA antigens among HTLV-I carriers differed from those in HTLV-I-negative control subjects, a comparison of HTLV-I carriers to patients with HTLV-I-related disease was conducted to identify HLA class II alleles among carriers that might be disease specific and predictive of subsequent development of ATL or HAM/TSP. HTLV-I carriers and patients with ATL and HAM/TSP were DNA typed for HLA class II, DRB1 and DQB1 alleles (Table 2).

Since the HTLV-I carriers had been previously serotyped, there was an opportunity to directly compare serotyping with DNA typing for the significant alleles, DR15 (DRB1*1501) and DQ1 (DQB1*0501,*0502,*05031,*0602,*0605,*0609). For HLA DR15, there was agreement between the two methods for each subject except one. Serologic typing misclassified DRB1*1601 (DR16) as DR15, both splits of the parent antigen DR2. For HLA DQ1, there was agreement between the two methods for each subject except one and another with missing results. Serologic typing misclassified DQB1*0201 (DQ2) as DQ1. DNA allele typing was able to exclude cross-reactive antibody patterns resulting from the serologic typing trays. The DNA allele typing was confirmed by linkage disequilibrium of DR-DQ alleles. Previous subjects with undetectable alleles with serologic typing were identifiable by use of DNA typing. None of the changes altered the statistical significance of the initial results shown in Table 1.

HLA class II allele frequencies were similar between HTLV-I carriers and pa-

Table 2. HLA-DRB1 allele frequencies (AF) of asymptomatic HTLV-I carriers (AC) compared with ATL and HAM/TSP*

HLA serotypes DR	DRB1	AC		ATL			HAM/TSP		
		(n = 43) [†] 86 alleles		(n = 49) 98 alleles		χ^2 [‡]	(n = 54) 108 alleles		χ^2 [‡]
		No.	AF, %	No.	AF, %		No.	AF, %	
1	*0101/2	8	9.3	6	6.1	0.7	11	10.2	0.0
2	*1501	22	25.6	20	20.4	0.7	11	10.2	8.1 ¹
	*1601	1	1.2	0	0.0	1.5	0	0.0	1.6
	*1602	0	0.0	1	1.0	1.3	2	1.9	2.4
3	*0301	6	7.0	7	7.1	0.0	12	11.1	1.0
	*0302	3	3.5	5	5.1	0.3	4	3.7	0.0
	*0303	0	0.0	0	0.0		2	1.9	2.4
4	*0401	1	1.2	0	0.0	1.5	1	0.9	0.0
	*0405	2	2.3	0	0.0	3.1	2	1.9	0.1
5	*1101	10	11.6	14	14.3	0.3	4	3.7	4.5 ²
	*1102	2	2.3	3	3.1	0.1	7	6.5	2.0
	*1103	0	0.0	1	1.0	1.3	0	0.0	
	*1105	0	0.0	0	0.0		2	1.9	2.4
	*1116	0	0.0	0	0.0		1	0.9	1.2
	*1201	3	3.5	2	2.0	0.4	2	1.9	0.5
6	*1301	4	4.7	9	9.2	1.5	6	5.6	0.1
	*1302	0	0.0	2	2.0	2.5	4	3.7	4.6 ³
	*1303	0	0.0	3	3.1	3.8	3	2.8	3.6
	*1304	0	0.0	1	1.0	1.3	1	0.9	1.2
	*1305/6	0	0.0	0	0.0		1	0.9	1.2
	*1308	0	0.0	0	0.0		1	0.9	1.2
	*1312	2	2.3	3	3.1	0.1	0	0.0	3.3
	*1317	0	0.0	0	0.0		1	0.9	1.2
	*1318	0	0.0	1	1.0	1.3	0	0.0	
	*1401	2	2.3	3	3.1	0.1	0	0.0	3.3
	*1404	0	0.0	0	0.0		2	1.9	2.4
	*1415	0	0.0	1	1.0	1.3	0	0.0	
7	*0701	8	9.3	9	9.2	0.0	14	13.0	0.7
8	*0801	2	2.3	0	0.0	3.1	0	0.0	3.3
	*0802	7	8.1	6	6.1	0.3	5	4.6	1.0
	*0803	0	0.0	0	0.0		1	0.9	1.2
	*0805	1	1.2	0	0.0	1.5	0	0.0	1.6
	*0806	0	0.0	1	1.0	1.3	3	2.8	3.6
9	*0901	0	0.0	0	0.0		1	0.9	1.2
10	*1001	2	2.3	0	0.0	<u>3.1</u>	4	<u>3.7</u>	<u>0.3</u>
						32.4 ⁵	61.2 ⁴		

*ATL = adult T-cell leukemia/lymphoma; HAM/TSP = HTLV-I-associated myelopathy/tropical spastic paraparesis.

[†]Unable to type DNA in two samples.

[‡]¹*P* = .005; ²*P* = .033; ³*P* = .13 by Fisher's exact test; ⁴*P* = .0014 by omnibus [G] test (32 *df*); and ⁵not significant.

tients with ATL with no significant differences (Tables 2 and 3). In contrast, there were two DRB1 alleles with significantly greater frequency among HTLV-I carriers than patients with HAM/TSP, DRB1*1501 (OR = 3.0; 95% CI = 1.3–7.4) and DRB1*1101 (OR = 3.4; 95% CI = 0.94–15.4). One DQB1 allele had significantly greater frequency among HTLV-I carriers than patients with HAM/TSP, DQB1*0602 (OR = 3.0; 95% CI = 1.5–6.4).

Comparisons of alleles between patients with ATL and HAM/TSP (Table 4)

revealed that two DRB1 alleles had greater frequency among ATL, DRB1*1101 (OR = 4.3; 95% CI = 1.3–18.6) and DRB1*1501 (OR = 2.3; 95% CI = 1.0–5.5). The HLA class II allele, DQB1*0602 was also significantly increased in frequency among patients with ATL compared with patients with HAM/TSP (OR = 2.3; 95% CI = 1.1–4.7). The haplotypes DRB1*1101-DQB1*0301 and DRB1*1501-DQB1*0602 were significantly increased (*P* = .01 and *P* = .04, respectively) among patients with ATL compared with HAM/TSP. The haplotype

Table 3. HLA-DQB1 allele frequencies (AF) of asymptomatic HTLV-I carriers (AC) compared with ATL and HAM/TSP*

HLA serotypes		AC		ATL			HAM/TSP		
		(n = 43)† 86 alleles		(n = 49) 98 alleles			(n = 54) 108 alleles		
		No.	AF, %	No.	AF, %	χ ²	No.	AF, %	χ ² ‡
DQ	DQB1								
1	*0501	14	16.3	10	10.2	1.5	16	14.8	0.1
	*0502	2	2.3	2	2.0	0.0	3	2.8	0.04
	*05031	0	0.0	1	1.0	1.3	2	1.9	2.4
	*0602	31	36.0	29	29.6	0.9	17	15.7	10.6 ¹
	*0603	1	1.2	5	5.1	2.5	4	3.7	1.3
	*0604	0	0.0	2	2.0	2.5	2	1.9	2.4
	*0605	1	1.2	2	2.0	0.2	1	0.9	0.3
	*0609	0	0.0	0	0.0		1	0.9	1.2
2	*0201/2	15	17.4	19	19.4	0.1	28	25.9	2.0
3 (7)†	*0301	15	17.4	20	20.4	0.3	28	25.9	2.0
	*0302	3	3.5	0	0.0	4.6 ²	1	0.9	1.6
	*03032	0	0.0	2	2.0	2.5	0	0.0	
4	*0402	4	4.7	6	6.1	0.2	5	4.6	0.0
						16.6 ⁴			23.9 ³

*ATL = adult T-cell leukemia/lymphoma; HAM/TSP = HTLV-I-associated myelopathy/tropical spastic paraparesis.

†Unable to type DNA in two samples. Number in () is split of parent serotype.

‡¹*P* = .001; ²*P* = .10 by Fisher's exact test; ³*P* = .02 by omnibus [G] test (12 *df*); and ⁴not significant.

DRB1*1501-DQB1*0602 was also significantly increased (*P* = .005) among HTLV-I carriers compared with patients with HAM/TSP. Several alleles were increased in frequency among HAM/TSP compared with HTLV-I carriers and ATL; however, they were not statistically significant.

HLA Among ATL and HAM/TSP in Jamaica and Japan

To determine whether these HLA alleles identified in other populations would confer susceptibility to HTLV-I and related disease, a comparison was made with findings reported among HTLV-I carriers and patients with HAM/TSP and ATL in Japan (Table 4) (26). Although the candidate alleles were lower in frequency among the Japanese population compared with Jamaica, DRB1*1501 and DQB1*0602 were significantly increased among ATL compared with HAM/TSP patients and among HTLV-I carriers compared with HAM/TSP in both populations. The increased frequency of the DRB1*1101 allele among ATL compared with HAM/TSP patients was unique to Jamaica.

Discussion

Among HTLV-I carriers, only a small percentage (1%–5%) of individuals de-

velop the HTLV-I related diseases, ATL or HAM/TSP (6,27). The major determinants of disease progression are not yet defined. On the basis of our results, it is reasonable to state that host immunogenetic factors, reflected by HLA background, are important determinants of HTLV-I disease outcome. The candidate, HLA class II genes have a significant role in regulating the immune response to infection and malignant transformation due to their function in presenting viral and other antigenic peptides to T lymphocytes (28). The immunologic status of patients with ATL and HAM/TSP are distinct (29,30), with both diseases rarely occurring in the same individual (31). Among carriers, those who develop ATL most

likely acquire genetic defects in various viral (32) and tumor suppressor genes (33,34) with progressive loss of immunologic control, resulting in malignant transformation. On the other hand, evidence suggests that HTLV-I carriers who subsequently develop HAM/TSP have an activated immune response with central nervous system damage mediated by high levels of HTLV-I-specific cytotoxic T lymphocytes (35). Other important determinants of disease outcome include age at time of infection and route of virus transmission.

Several HLA class II alleles were associated with HTLV-I infection in the Jamaican population. The HTLV-I carrier state was distinguished from HTLV-I negative control subjects by two significant antigens, DR15 and DQ1. These antigens were in linkage disequilibrium and DR15 was always detected in the presence of DQ1. DQ1 was significant, even after adjustment for multiple comparisons of antigens with the conservative Bonferroni's correction. Study participants were carefully selected to prevent obscuring true HLA associations ascribed to HTLV-I infection from genetic differences, because populations in Jamaica and other parts of the Caribbean are known to be heterogeneous, consisting of different ethnic groups with racial admixture of native Amerindian, Caucasian, and African descent (36). In our assessment of the immune response to virus, using antibody titers, there were no differences in antibody titer levels among carriers with and without DR15 and/or DQ1. The corresponding alleles by DNA typing, DRB1*1501 and DQB1*0602, have been associated with the low immune response profile characteristic of patients with ATL (37).

Table 4. HLA-DRB1 and -DQB1 allele frequencies (AF) among ATL and HAM/TSP patients in Jamaica and Japan*

HLA allele	Jamaica					Kyushu, Japan (26)				
	ATL (98 alleles)		HAM/TSP (108 alleles)		<i>P</i>	ATL (212 alleles)		HAM/TSP (236 alleles)		<i>P</i>
	No.	AF, %	No.	AF, %		No.	AF, %	No.	AF, %	
DRB1										
*1101	14	14.3	4	3.7	.006	4	1.9	6	2.5	.75
*1501	20	20.4	11	10.1	.04	15	7.1	3	1.3	.002
DQB1, *0602	29	29.6	17	15.7	.017	15	7.1	3	1.3	.002

*ATL = adult T-cell leukemia/lymphoma; HAM/TSP = HTLV-I-associated myelopathy/tropical spastic paraparesis.

In a previous report, HLA class I alleles, A36 and B18, were found to be increased among patients with ATL compared with HTLV-I carriers in a population of African descent, however, no differences in serologically determined class II alleles were identified (38). Similarly, there were no significant differences in class II allele frequencies between ATL patients and HTLV-I carriers in the current study. There were corresponding differences in allele frequencies (DRB1*1101, DRB1*1501, and DQB1*0602) between these groups and patients with HAM/TSP, suggesting that the alleles identified were as follows: 1) representative of the carrier state and infection or 2) indicative of carriers at risk for progression to ATL but not HAM/TSP. We found several alleles with increased frequency among patients with HAM/TSP although they did not approach statistical significance, possibly due to our modest sample size. In contrast to these findings, several class II alleles have been shown to be increased among patients with HAM/TSP that are unique to endemic Japanese populations (37).

A comparison of results with those from Japan revealed that similar patterns of disease association were identified for DRB1*1501 and DQB1*0602 but not for DRB1*1101. The DRB1*1101 allele had a lower frequency (2.1%) in the Japanese population compared with West African (Senegalese) and North American Blacks (9.4% and 8.2%, respectively) in other reports (39). On the other hand, the DR15-DQ1 haplotype has been associated with ATL and HAM/TSP in Japan (37). With DNA typing, ATL was characterized by DRB1*1501-DQB1*0602, while HAM/TSP was characterized by DRB1*1502-DQB1*0601 (37). Thus, this haplotype may prove useful as a disease marker across racial groups, since it is a common haplotype among Asians, Caucasians, and African lineages (40). Together, these observations suggest that there may be common haplotypes shared by individuals at risk for HTLV-I-related disease. Additionally, this same haplotype has been identified among Hispanic populations at risk for human papillomavirus type 16-associated cervical neoplasia (41), suggesting that a similar immunogenetic background may be implicated in immunologic control with several viral-related cancers. These intriguing observations re-

quire further investigation. Finally, this exploratory analysis will require subsequent confirmation with additional studies of association or family studies of HTLV-I-related disease.

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Notes

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